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The breast cancer susceptibility gene, *BRCA2*: at the crossroads between DNA replication and recombination?

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The identification and cloning of the familial breast cancer susceptibility gene, *BRCA2*, has excited much interest in its biological functions. Here, evidence is reviewed that the protein encoded by *BRCA2* has an essential role in DNA repair through its association with mRad51, a mammalian homologue of bacterial and yeast proteins involved in homologous recombination. A model is proposed that the critical requirement for BRCA2 in cell division and the maintenance of chromosome stability stems from its participation in recombinational processes essential for DNA replication.

Keywords: BRCA2; DNA replication; homologous recombination; cancer

1. BREAST CANCER SUSCEPTIBILITY GENES AND CANCER PREDISPOSITION

Inherited mutations in the *BRCA2* gene predispose humans to familial, early-onset breast cancer (Wooster *et al.* 1994, 1995; Tavtigian *et al.* 1996). The gene was first identified by a positional cloning approach through the analysis of families exhibiting an increased susceptibility. Moreover, founder mutations in the *BRCA2* gene associated with increased cancer susceptibility have been identified in several geographically or ethnically restricted human populations (for example, Gudmundsson *et al.* 1996; Johannesdottir *et al.* 1996; Neuhausen *et al.* 1996; Thorlacius *et al.* 1996). In contrast, mutations in *BRCA2* do not appear to be a feature of sporadic breast cancer (reviewed in Rahman & Stratton 1998).

BRCA2 is not simply a breast cancer susceptibility gene. The spectrum of cancer predisposition associated with *BRCA2* mutations has not fully been characterized. It is already clear, for example, that *BRCA2* mutation carriers are also susceptible to familial ovarian cancer (Wooster *et al.* 1994; Tavtigian *et al.* 1996). Cancers of the prostate, pancreas and male breast may also be associated with *BRCA2* mutations (Thorlacius *et al.* 1996). The occurrence of thymic lymphomas in currently available mouse models for *BRCA2* deficiency (Connor *et al.* 1997; Friedman *et al.* 1998), to be discussed elsewhere in this paper, deserves mention. Although there does not appear to be an excess risk of lymphoma in human *BRCA2* mutation carriers, the incidence of *BRCA2* mutations in sporadic cases of lymphoma remains to be evaluated.

Despite the wide scientific and public interest engendered by the cloning of *BRCA2*, little information has been gleaned merely by analysis of the gene's sequence. It encodes a large protein of 3418 amino acids in humans, which bears no significant resemblance to molecules of known function. The only remarkable feature is the presence of a cluster of eight repeated sequences, the so-called BRC repeats (Bork *et al.* 1996), located within *BRCA2* exon 11 (figure 1). The sequence of the BRC repeats is highly conserved between several species of mammals, although the intervening sequences are not (Bignell *et al.* 1997). Viewed in the light of the generally limited (*ca.* 60%) homology between murine and human BRCA2 proteins (Sharan & Bradley 1997), this suggests that the BRC repeats may have a conserved and essential role in the function of BRCA2. It may be significant that the *BRCA2* mutations associated with familial ovarian cancer tend to cluster within the BRC repeat region encoded by exon 11 (Gayther *et al.* 1997). It must be noted, however, that these are truncating mutations, making it difficult to explain why truncations positioned more 5′ in the gene, which also result in the loss of BRC repeats, are not associated with a similar phenotype.

Positional cloning has also identified another breast cancer susceptibility gene, *BRCA1*, which in humans encodes a protein of 1863 amino acids (Miki *et al.* 1994). It is important to emphasize that despite the similarity in their acronyms, the molecules themselves are highly distinct in sequence (figure 1). There is much circumstantial evidence, however, to suggest some commonality in their functions. Mutations in either gene confer a marked increase in breast cancer risk in humans. Targeted truncations in the murine homologues of *BRCA1* (Gowen *et al.* 1996; Hakem *et al.* 1996; Liu *et al.* 1996; Ludwig *et al.* 1997) or *BRCA2* (Ludwig *et al.* 1997; Sharan *et al.* 1997; Suzuki *et al.* 1997) result in either early embryonic lethality at day 7–9 or, if positioned more carboxyl-terminally in *BRCA2* (Connor *et al.* 1997; Friedman *et al.* 1998), severe embryonal growth retardation, perinatal lethality and the development of thymic tumours in surviving adults. Both genes encode nuclear proteins that are highly expressed during

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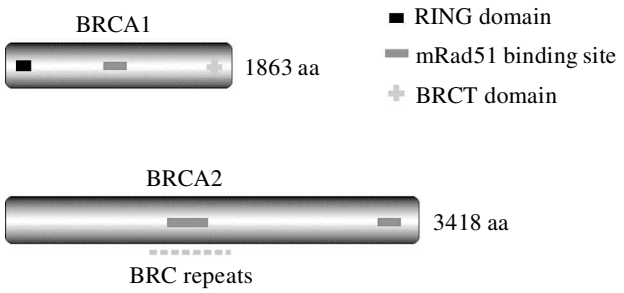

Figure 1. Shows a schematic representation of the human BRCA1 and BRCA2 proteins. Known structural features are marked, but are not drawn to scale. The RING and BRCT domains in BRCA1 are putative protein–protein interaction motifs. The Rad51 binding sites in both proteins, and the structural motifs found in BRCA2, are discussed in §§ 1 and 3.

the S phase of the cell cycle (Rajan *et al.* 1996; Bertwistle *et al.* 1997; Jin *et al.* 1997). Finally, there is evidence that BRCA1 and BRCA2 proteins co-localize to subnuclear structures in mitotic and meiotic cells, and may even physically associate at low stoichiometry (Chen *et al.* 1998*c*).

For both *BRCA1* and *BRCA2*, inheritance of a single defective allele is sufficient to confer cancer predisposition, but tumours isolated from mutation carriers almost always exhibit a loss of heterozygosity (Collins *et al.* 1995; Gudmundsson *et al.* 1995). By contrast, there are no reports of tumour predisposition in mice heterozygous for targeted mutations in *BRCA1* or *BRCA2*. No satisfactory explanation is yet available for this species-specific difference in the phenotype of *BRCA2* heterozygotes.

2. *BRCA2* AND DNA REPAIR

There is little in the nucleotide sequence of *BRCA2* that is suggestive of its biological function. The first insights have therefore emerged from studies of mice harbouring targeted mutations in the murine homologue of the *BRCA2* gene (hereafter termed *Brca2* in accordance with the accepted nomenclature). An essential role in embryonal cellular proliferation and as a consequence, in intrauterine viability, has been inferred from the marked growth retardation and early embryonal lethality apparent in animals homozygous for truncations in *Brca2* exon 10 (Ludwig *et al.* 1997; Sharan *et al.* 1997; Suzuki *et al.* 1997). Perhaps more amenable to further study, a role for *Brca2* in the cellular response to DNA damage has been postulated from the observation that it associates with mammalian (m)Rad51 (Chen *et al.* 1998*b*; Mizuta *et al.* 1997; Sharan *et al.* 1997; Wong *et al.* 1997), a homologue of the bacterial protein RecA, which is known to function in the repair of DNA double-strand breaks (DSBs) by genetic recombination. Consistent with this notion, murine blastocysts homozygous for a *Brca2* exon 10 truncation exhibit X-ray sensitivity (Sharan *et al.* 1997), as do fibroblasts harbouring a truncation in the 3′ region of exon 11 (Connor *et al.* 1997).

Although these observations hint at a role for Brca2 in DNA repair, they admit of several explanations. The cellular response to DNA damage (reviewed in Friedberg *et al.* 1995) involves the activation of cell-cycle checkpoints to prevent the replication of damaged DNA templates, the recruitment of the machinery for DNA repair and sometimes, the induction of apoptosis in the face of overwhelming, irreparable genetic damage. Thus, an increased sensitivity to X-rays is only indicative of a potential dysfunction in any of the cellular responses to DNA damage, and not necessarily of defective DNA repair *per se*.

It is therefore important that checkpoint activation and apoptosis appear to be largely intact in fibroblasts and lymphocytes isolated from mice harbouring a targeted truncation at residue 1492 in *Brca2* exon 11 (termed the *Brca2*^{Tr} allele) (Patel *et al.* 1998). Brca2$^{Tr/Tr}$ cells exhibit arrest in the G1 and G2/M phases of the cell cycle following exposure to X-rays or UV light, and abrogate DNA synthesis after treatment with hydroxyurea. Moreover, even small doses of X-rays can induce the apoptotic death of Brca2$^{Tr/Tr}$ cells just as efficiently as in wild-type cells. Thus, these observations indicate that Brca2 has an essential function in some aspect of the DNA damage response that is distinct from participation in checkpoint enforcement or apoptosis. Since the Brca2^{Tr} allele leaves the exons encoding residues 1–1492 intact, a role for these amino-terminal domains in checkpoints or apoptosis is not, of course, excluded by these findings (Patel *et al.* 1998). It is noteworthy in this context that a *Brca1* exon 11 in-frame deletion gives rise to abnormalities in the G2/M checkpoints (Xu *et al.* 1999), although the significance of this observation to Brca2 function remains to be ascertained.

The spectrum of genotoxin sensitivity exhibited by Brca2$^{Tr/Tr}$ cells is consistent with a defect in DNA repair by homologous recombination (Patel *et al.* 1998). Like Brca2$^{Tr/Tr}$ cells, yeast mutants in the *RAD52* epistasis group of genes (including *RAD51*, *RAD52*, *RAD55*, *RAD57*, *RAD59*, *MRE11* and *Xrs2*) involved in the repair of DSBs by homologous recombination generally exhibit cross-sensitivity to X-rays, UV light and monofunctional alkylating agents. Similar sensitivities are observed in vertebrate cells deficient in *Rad54* (Bezzubova *et al.* 1997; Essers *et al.* 1997), as well as in *Rad51*-related genes (Jones *et al.* 1987; Lim & Hasty 1996; Tsuzuki *et al.* 1996; Liu *et al.* 1998; Sonoda *et al.* 1998).

Strikingly, Brca2$^{Tr/Tr}$ cells spontaneously accumulate numerous chromosomal aberrations (Patel *et al.* 1998) during passage in culture (figure 2). The aberrations include chromatid and chromosome breaks, and the formation of structures termed tri-radial and quadri-radial chromosomes previously noted in the human genetic disease Bloom syndrome (German 1993). These latter abnormalities (figure 2) reflect defective chromatid exchange during homologous recombination in mitotic cells, with tri-radials (Y-shaped chromosome structures with three arms) indicative of incomplete separation following isochromatid exchange and quadri-radials (star-shaped structures with four arms) arising from a flawed exchange between chromosomes. More recently, similar abnormalities have been described in *Brca1* exon-11-deficient murine cells (Xu *et al.* 1999). These observations demonstrate that Brca1 deficiency or Brca2 deficiency in mouse models may give rise to a phenotype reminiscent of other human diseases (table 1) in which chromosomal instability is associated with cancer

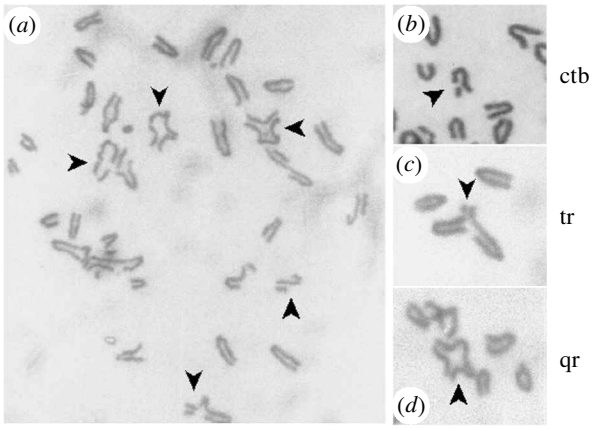

Figure 2. Chromosomal abnormalities that spontaneously accumulate in cultures of murine fibroblasts homozygous for a targeted truncation in the *Brca2* gene. Note the high frequency of the aberrations, and the occurrence of (*a*) chromatid (ctb) and chromosome breaks, (*b*) tri-radial (tr), and (*c*) quadri-radial (qr) chromosomes. Reproduced from Patel *et al*. 1998, with the permission of Cell Press.

predisposition. More specifically, they suggest that some features of this phenotype may arise from a defect in the ability to correctly execute or regulate DNA repair by homologous recombination.

3. DNA REPAIR BY HOMOLOGOUS RECOMBINATION

The mechanism for homologous recombination is most clearly understood in *Escherichia coli*. The bacterial RecA protein plays a central role in the process (reviewed in Roca & Cox 1997). It recognizes and aligns homologous regions in the two DNA molecules that are to undergo recombination, and mediates the strand exchange reaction, which generates a crossover between them. A number of additional proteins are necessary to initiate, regulate and complete the reactions catalysed by RecA. The RecBCD protein complex, which includes DNA helicase, ATPase, exo- and endonuclease activities, acts at DSBs to generate a stretch of single-strand DNA that serves as a substrate for homologous strand pairing by RecA. The single-strand DNA substrate is coated by RecA polymers to form a nucleoprotein filament. Filament assembly is regulated by the RecFOR proteins and the single-strand binding protein SSB. Finally, the RuvA-C proteins act to resolve the recombination intermediates formed by the action of RecA through effects on branch migration, and on Holliday junction resolution by endonucleolytic cleavage.

Homologous recombination in *E. coli* has classically been studied as a mechanism for the generation of diversity during conjugation, a process for which the RecA pathway is essential. There is much evidence, however, that the pathway also plays an important role in DNA repair, and may in particular be an effective response to DNA strand gaps or DSBs (Meselson & Radding 1975; West *et al*. 1981; Szostak *et al*. 1983). This is certainly true in yeast, where homologous recombination is the major pathway for the repair of DSBs. It has been asserted that, in contrast, mammalian cells repair DSBs primarily by non-homology-dependent mechanisms such as non-homologous end joining (NHEJ) (reviewed in Friedberg *et al*. 1995; Jackson & Jeggo 1995). The evidence to support this assertion is largely indirect, coming from studies in which integration, as opposed to homologous recombination, of transfected DNA predominates in mammalian cells. More recent work, however, challenges the notion that homologous recombination is a minor or unimportant pathway for DSB repair in mammalian cells. DSBs experimentally induced into mammalian chromosomes by the activity of rare-cutting endonucleases enhance homologous recombination by two to three orders of magnitude (Rouet *et al*. 1994). As many as 30–50% of DSBs introduced in this way at direct repeat sequences are resolved by homology-directed repair mechanisms (Liang *et al*. 1998). Indeed, although rodent cells deficient in the major NHEJ pathway dependent on the *xrcc4–7* genes are highly sensitive to ionizing radiation during G1, they are relatively resistant late in the S phase or in G2, when homologous recombination can occur between replicated DNA strands (Stamato *et al*. 1988; Whitmore *et al*. 1989; Cheong *et al*. 1994). A similar phenotype is apparent in Ku70-deficient or RAD54-deficient avian cells, defective in NHEJ or recombination mechanisms, respectively (Takata *et al*. 1998). Collectively, these observations suggest that homologous recombination may be of particular importance for DNA repair during DNA replication in mammalian cells.

Table 1. *Genetic diseases where aberrations in chromosome structure are associated with cancer predisposition*

Bloom syndrome	*Brca1* deficiency (mouse model)
Fanconi anaemia	
Ataxia–telangiectasia	*Brca2* deficiency (mouse model)
Nijmegen breakage syndrome	

It should be emphasized that, quite apart from its potential role in DNA replication and/or repair in mitotic cells, homologous recombination initiated at DSBs is essential for the normal completion of meiosis (Haber 1997; Keeney *et al*. 1997). It is therefore provocative that BRCA2 (as well as BRCA1 and mRad51) co-localize to the synaptonemal complexes formed at meiotic chromosomes (Chen *et al*. 1998*c*). Indeed, the reproductive sterility and germ cell abnormalities observed in *Brca2*-deficient mice (Connor *et al*. 1997; Friedman *et al*. 1998) are consistent with defective meiosis, although this has yet to be firmly established.

In eukaryotes, Rad51 plays a central role in homologous recombination analogous to that of RecA. RAD51 was first identified in yeast (Shinohara *et al*. 1992*b*), as was its meiosis-specific homologue; Dmc1 (Bishop *et al*. 1992). Mammalian homologues of both proteins exist. Interestingly, however, at least five further mRad51-like molecules are also present in vertebrate cells, including the products encoded by the *xrcc2* and *xrcc3* genes isolated by complementation of X-ray-sensitive CHO cell mutants (Cartwright *et al*. 1998; Liu *et al*. 1998), as well as *Rad51B*, *Rad51C* and *Rad51D* (Albala *et al*. 1997; Dosanjh *et al*. 1998; Pittman *et al*. 1998). The reason for the multiplicity of mRad51-like molecules in vertebrates is uncertain. Yet there appears to be little redundancy for those functions of mRad51 that are essential for cell division and viability,

since its targeted disruption is lethal both to murine and avian cells (Lim & Hasty 1996; Tsuzuki *et al*. 1996; Sonoda *et al*. 1998). In contrast, mutations in yeast *RAD51* engender defective homologous recombination and meiosis, but do not cause lethality (Shinohara *et al*. 1992*a*).

Despite this important phenotypic difference, the available biochemical evidence suggests a great similarity between the function of yeast and mammalian Rad51 molecules in homologous recombination. Both proteins form helical filaments on DNA in a manner similar to bacterial RecA (Ogawa *et al*. 1993; Sung & Robberson 1995). In concert with Rad52 (Sung 1997; Benson *et al*. 1998; New *et al*. 1998; Shinohara *et al*. 1998), they mediate homologous pairing between DNA strands (Sung 1994; Sung & Robberson 1995; Baumann *et al*. 1997) to promote a strand exchange reaction. The single-strand DNA binding protein, RPA, considerably enhances this reaction, which is associated with (Sung 1994; Baumann *et al*. 1997; Gupta *et al*. 1997), but may not depend on (Sung & Stratton 1996), a DNA-dependent ATPase activity intrinsic to yeast and mammalian Rad51.

Differences between RecA and eukaryotic Rad51 are also considerable. The strand exchange reaction promoted by the eukaryotic enzymes is far weaker than that mediated by RecA (Sung 1994; Baumann *et al*. 1997). Moreover, their ATPase activity is some 100-fold less than that of RecA (Gupta *et al*. 1997). These biochemical measurements in *in vitro* experimental systems hint at the necessity for additional eukaryotic proteins to modulate the activity of Rad51 and to regulate—in a manner not required in *E. coli*—the *in vivo* progress and extent of the recombination reaction. It is in this context that the physical association of mRad51 with tumour suppressor proteins such as p53 (Sturzbecher *et al*. 1996), BRCA1 (Scully *et al*. 1997) and BRCA2 (Chen *et al*. 1998*b*; Mizuta *et al*. 1997; Sharan *et al*. 1997; Wong *et al*. 1997) may be of considerable importance to its essential role in cell division and viability.

Given the central role established for mRad51 in homologous recombination, it is striking that several lines of circumstantial evidence indicate a shared function with Brca2. The genes encoding these proteins show a similar expression pattern in mammalian cells, with low levels expressed in many tissues and with particularly high levels found in the thymus and testes. Expression is restricted, as in the case of *BRCA1*, primarily to the S phase of the cell cycle. As mentioned earlier, mRad51 and Brca2 are physically associated at relatively high stoichiometry. Co-localization can be demonstrated to nuclear foci in mitotic cells, and to synaptonemal complexes in meiotic cells. Finally, like $Brca2^{Tr/Tr}$ cells, Rad51-deficient vertebrate cells spontaneously accumulate chromosomal abnormalities and are unable to maintain continued division in culture.

4. *BRCA2*: AT THE CROSSROADS BETWEEN DNA REPLICATION AND RECOMBINATION?

What are the common biological functions that may underlie the essential requirement for Rad51 and Brca2 in cell division and chromosome stability? Paradigms from work on *E. coli* suggest that they may be related to the necessity for RecA-catalysed strand exchanges to enable the error-free resumption of DNA replication when replication fork progression is stalled (reviewed in West *et al*. 1981; Kuzminov 1995; Kogoma 1996, 1997; Cox 1997). Several lesions encountered by a replication fork could lead to its collapse, including strand gaps, base adducts causing template distortion, or DSBs. In these situations, replication restart in *E. coli* is dependent on the use of recombinational mechanisms to bypass the lesion and enable origin-independent reinitiation of the replication fork from recombination intermediates (for example, Kogoma 1996; Liu *et al*. 1999). Failure to carry out replication restart would result not only in defective DNA synthesis and cell proliferation, but could also provoke the occurrence of discontinuities in replicated chromosomes.

An important element in this scheme is the notion that replication forks are frequently stalled during normal cell division. Conditions that precipitate stalling could conceivably arise in many different ways. These may include base lesions created by DNA modification (reviewed in Lindahl 1993, 1996) through hydrolysis, oxidation and other reactions, UV-induced strand alterations, base mismatches caused by replication errors, or simply by replication fork encounter with DNA-bound proteins. No direct measurement of the extent of replication stalling during normal growth is available. In *E. coli* RecBCD or RecARecD mutants, stalling of replication forks results in the generation of DSBs (Michel *et al*. 1997; Seigneur *et al*. 1998). It is provocative that in these strains, DSBs spontaneously accumulate to high levels during normal growth indicative of the considerable frequency of replication fork stalling which must occur during DNA replication. Moreover, DSB accumulation is even further elevated in this background when DNA replication is impeded by disruption of replicative helicases (Michel *et al*. 1997), emphasizing the role of homologous recombination in the repair of DNA damage induced by defective replication.

To what extent can the paradigms developed in work on bacteria be extended to eukaryotes? There is limited but intriguing evidence that homologous recombination is stimulated by, and necessary for, eukaryotic DNA replication. Recombination intermediates representing unresolved Holliday junctions accumulate spontaneously during the S phase of the cell cycle in synchronously dividing yeast cells in the absence of exogenously induced DNA damage, suggesting that recombination operates to repair replication-associated lesions (Zou & Rothstein 1997). Indeed, mutations affecting components of the replicative machinery increase the level of recombination intermediates detected in this system. This may provide some mechanistic substance to the oft-noted observation that in mammalian cells, mutations in DNA polymerases, ligases or helicases such as the RecQ homologue altered in Bloom syndrome, greatly stimulate genetic exchange between sister chromatids as detected by differential BrdU labelling of newly replicated DNA strands (reviewed in Rothstein & Gangloff 1995).

In the model proposed here, in which recombinational processes dependent on Rad51 and Brca2 are required for normal DNA replication, the proliferative impediment apparent in Rad51-deficient avian cells and in $Brca2^{Tr/Tr}$ mouse fibroblasts is of particular interest. Rad51 deletion results in a gradual loss of replicative capacity (Sonoda *et al*. 1998), with progressive accumulation in the G2/M phases of the cell cycle consistent with arrest at the check-

points that monitor the completeness of DNA replication. $Brca2^{Tr/Tr}$ fibroblasts also display a progressive impediment to their capacity to undergo cell division (Patel *et al*. 1998). When freshly isolated from murine embryos, they are roughly similar in their proliferative competence to wild-type or heterozygous control cells. A severe proliferative defect becomes apparent, and progressively worsens, the more often the cells are passed in culture, accompanied by increasing G1 and G2/M phase arrest. These phenotypes of Rad51-deficient and $Brca2^{Tr/Tr}$ cells are reminiscent of RecF pathway mutants of *E. coli*, which also exhibit an inability to sustain ongoing rounds of DNA replication even in the absence of exogenously induced DNA damage (Courcelle *et al*. 1997).

Studies of co-localization also lend support to the idea that mRad51 and BRCA2 participate in repair processes associated with DNA replication. Nuclear foci containing mRad51, BRCA1 and BRCA2 are formed during late S and G2 (Chen *et al*. 1998*c*). Following exposure to hydroxyurea, which depletes nucleotide pools required for DNA replication, BRCA2-containing foci costain with PCNA (Chen *et al*. 1998), suggesting localization at replication foci. These observations are consistent with a model in which the activity of BRCA2 and Rad51 is necessary to overcome the replication fork stalling at strand gaps induced by hydroxyurea. It is currently unclear if BRCA2 or Rad51 interact directly with components of the eukaryotic DNA replication machinery or with the accessory molecules involved in regulating the epigenetic effects associated with DNA replication. This is a prediction of the model proposed here, and is likely to be a worthwhile focus for further analysis.

The model proposed here for the function of BRCA2–mRad51 in eukaryotic DNA replication has important implications for the role of BRCA2 mutations in cancer predisposition. Defects in components of the replication machinery have clearly been associated with a mutator phenotype in eukaryotes (Chen *et al*. 1998*a*), suggesting that BRCA2-deficient cells may also sustain elevated mutation rates even without exogenously induced DNA damage. The replication defect and chromosomal instability observed in Brca2-deficient cells at first glance seem at odds with the unrestrained proliferation associated with cancer. It can therefore be predicted that secondary mutations—incurred at a high frequency because of the mutator phenotype—must be selected during the process of transformation to yield cancer cells, which have in some way ameliorated or compensated for any underlying replication and/or repair defect (Lee *et al*. 1999). If this model is correct, the identification and analysis of genes that consistently undergo secondary mutation in BRCA2-deficient tumour cells are likely to provide valuable biological insights into the mechanism of replication-associated recombination in mammalian cells.

Over-reliance on analogies to the bacterial RecA pathway in interpreting or predicting the manner in which homologous recombination and replication may interact in mammalian cells is, of course, to be avoided. For a start, there are many significant differences between the activities of the enzymes involved. As discussed previously, mRad51 is far less proficient at promoting strand exchange, exhibits poor ATPase activity and appears to work with the opposite strand polarity, when compared to RecA. Eukaryotic DNA replication undoubtedly occurs in a more complicated molecular and cellular milieu than in prokaryotes, necessitating the action of additional molecules for which there may be no counterparts in the simpler system. For similar reasons, analogies to yeast may also be of limited value. Homologues to p53, BRCA1 or BRCA2—all known to interact with mRad51—do not appear to exist in yeast. Mammalian cells contain a multiplicity of Rad51-like proteins. Yeast *RAD52*, but not *RAD51*, *RAD55* or *RAD57*, appears to be essential for the formation of recombination intermediates associated with replication (Zou & Rothstein 1997). Many of these discrepancies will ultimately be resolved by biochemical studies to elucidate the activities of the mammalian enzymes involved in homologous recombination and to define their inter-molecular interactions, an effort now underway in several laboratories. Despite these limitations to a model for mRad51/Brca2 activity based on bacterial parallels, its formulation does provide a useful framework for further work in which informative predictions can be made for experimental analysis.

While this review is focused on the functions of BRCA2 in DNA repair in relation to its interaction with mRad51, it bears reiteration that this exceptionally large nuclear protein is very likely to have multiple functions within a cell that may or may not be relevant in this context. For example, functions in transcription activation (Chapman & Verma 1996; Fuks *et al*, 1998) and transcription-coupled DNA repair (Gowen *et al*. 1998) have been ascribed to both BRCA1 and BRCA2. The relationship of these putative roles to the phenotype of Brca2-deficient murine cells is uncertain, and their potential contribution to chromosome instability and cancer predisposition remains to be fully explored.

Finally, it remains unclear to what extent the study of BRCA2 will enlarge our understanding of breast cancer pathogenesis in general. Somatic BRCA2 mutations do not occur in non-familial breast cancers, which account for over 90% of incidence, undermining (but not entirely excluding) the conjecture that a common pathway involving the molecule will be dysfunctional in sporadic as well as familial tumours. Moreover, BRCA2 is widely expressed and appears to have important functions in cellular processes such as DNA repair and transcription apparently fundamental to all tissues. Why should BRCA2 mutations therefore result in predisposition to breast and ovarian cancer in particular? These are important gaps in our current understanding (Venkitaraman 1999), which cannot easily be resolved without invoking additional—and perhaps tissue-specific—functions for BRCA2 which remain to be identified.

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